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Fungal life in the extremely hypersaline water of the Dead Sea: first records

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The first report, to our knowledge, on the occurrence of filamentous fungi in the hypersaline (340 g salt l^{-1}) Dead Sea is presented. Three species of filamentous fungi from surface water samples of the Dead Sea were isolated: *Gymnascella marismortui* (Ascomycota), which is described as a new species, *Ulocladium chlamydosporum* and *Penicillium westlingii* (Deuteromycota). *G. marismortui* and *U. chlamydosporum* grew on media containing up to 50% Dead Sea water. *G. marismortui* was found to be an obligate halophile growing optimally in the presence of 0.5–2 M NaCl or 10–30% (by volume) of Dead Sea water. Isolated cultures did not grow on agar media without salt, but grew on agar prepared with up to 50% Dead Sea water. This suggests that they may be adapted to life in the extremely stressful hypersaline Dead Sea.

Keywords: fungi; obligate halophile; Dead Sea

1. INTRODUCTION

One of the most striking characteristics of life on earth is its rich variety (Wilson 1992). However, efforts to determine the extent of biotic diversity have long been inadequate (May 1988, 1995). This is especially true for distinctly speciose groups such as nematodes, arthropods and fungi (Hammond 1995), and for organisms from ecologically extreme environments.

In recent decades, researchers have paid attention to the underestimated but important role of fungi in the degradation of organic material in marine and hypersaline ecosystems. The level of degradative processes caused by fungi in the sea is not understood. Jones (1988) showed that in marine ecosystems, fungi are much more active biodegraders than was previously thought. To date, about 800 species of obligate marine fungi have been described, including species of Basidiomycota, Ascomycota, lichen-forming fungi, Deuteromycota and yeasts (Kohlmeyer & Kohlmeyer 1979; Hawksworth *et al*. 1995).

Reports of fungi from extremely hypersaline environments are very few. However, lack of representation in the literature may reflect not the inability of fungi to colonize these extreme environments, but rather the little effort that has been invested to find them (Javor 1989). The only report known to us on the isolation of a halophilic filamentous fungus from a hypersaline lake is the description of a *Cladosporium* sp. (Hyphomycetes, anamorphic Mycosphaerellaceae) on a submerged piece of pine wood in the Great Salt Lake, Utah (290–360 g l^{-1} salinity) (Cronin & Post 1977). In the same lake, non-filamentous 'fungi' from the genus *Thraustochytrium* were also found (Amon 1978; Brown 1990). In addition, a number of salt-tolerant fungi have been isolated from such sources as salted fish, seawater and desert soils (Andrews & Pitt 1987; Blomberg & Adler 1993; Adler 1996). Many fungi grow in the presence of very low water activities: *Aspergillus* species can grow down to an a_w of 0.70 and *Xeromyces bisporus* L. R. Fraser may grow at an a_w as low as 0.61 (Kushner 1978; Brown 1990).

The Dead Sea, located in the Syrian–African rift valley, on the border between Israel and Jordan, is one of the most saline lakes on earth (salinity about 340 g l^{-1}). The lake differs from other hypersaline lakes in the unique ionic composition of its waters, with concentrations of divalent cations (Mg, 40.7 g l^{-1}; Ca, 17 g l^{-1}) exceeding those of monovalent cations (Na, 39.2 g l^{-1}; K, 7 g l^{-1}). The major anions are Cl (212 g l^{-1}) and Br (5 g l^{-1}). The water activity in undiluted Dead Sea water was reported to be about 0.669 in 1979 (Krumgalz & Millero 1982), and today is probably even lower.

Since the discovery of life in the Dead Sea by B. Wilkansky (Benjamin Elazari-Volcani) in 1936 (Wilkansky 1936), the lake is known to be inhabited by several types of microorganisms. These include archaeal and eubacterial prokaryotes, unicellular green algae (*Dunaliella parva* Lerche), and possibly even protozoa (Elazari-Volcani 1940; Volcani 1944). Quantitative studies performed since 1980 have shown the lake to be a dynamic ecosystem. There are periods of dense blooms of algae (*D. parva*) and red halophilic Archaea, triggered by

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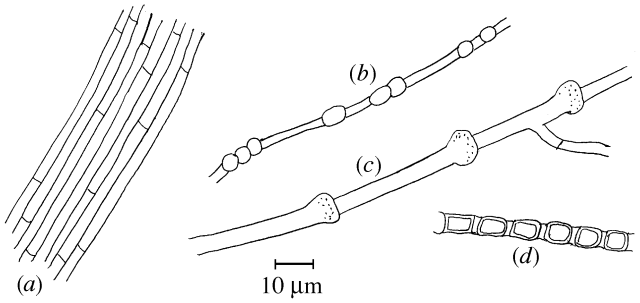

Figure 1. *Gymnascella marismortui*. (*a*) Mycelial cords; (*b*) chlamydospores; (*c*) nodulose hyphae; (*d*) arthroconidia.

dilution of the upper water layers by freshwater floods during rainy winters. Blooms alternate with long periods characterized by an almost total absence of life forms (Oren 1988, 1992, 1993).

No fungi have been recorded hitherto in the Dead Sea, with the possible exception of an osmophilic yeast, reported by Kritzman (1973), able to grow in a medium containing 15% glucose and 12% salt. Further details are lacking, and no cultures have been preserved. The paucity in biodiversity of the Dead Sea biota is probably determined by the high concentration of magnesium and calcium and the unique composition of the Dead Sea waters.

The present communication, we believe, presents the first description of filamentous fungi isolated from the Dead Sea water.

2. MATERIALS AND METHODS

Surface water samples were obtained from different sites of the Israeli Dead Sea shore, near Ein-Zukim, in January–November 1995. For the isolation of fungi, 2 ml of Dead Sea water was poured into a Petri dish and mixed by rotation with molten agar media. The following media were used: malt extract agar, malt extract agar with 20–50% (by volume) of Dead Sea water, and Czapek agar. All media were amended with tetracycline and streptomycin (100 $\mu g\,ml^{-1}$ each) to inhibit bacterial growth. After solidification of the agar, plates were incubated at 26–28 °C and 37 °C for 30 d each. Fungal colonies obtained were transferred to tubes with agar media for identification and storage. All isolates were grown on artificial seawater medium (RILA products, Teaneck, NJ, USA) glucose–peptone–yeast extract agar (Rohrmann *et al*. 1992) with final salinities of 3, 15 and 17.5%, or on media prepared in different dilutions of Dead Sea water, as indicated, at different temperatures (22, 27, 35 and 37 °C).

Fungi were also recovered from the deeper waters of the Dead Sea. Samples were collected on 8 October 1996 from the centre of the lake, about 8 km north-east of Ein Gedi, by means of Go-Flo sampling bottles, or by pumping through a hose. Samples were transferred to sterile bottles. Triplicate portions of 2 ml of water from different depths were added to 20 ml glass scintillation vials, containing 0.2 g of malt extract and 1 g of sucrose in 8 ml of distilled water, sterilized by autoclaving, to give a final Dead Sea water concentration of 20% (by volume). The bottles were incubated at 25 °C, and the occurrence of growth was followed for 30 d.

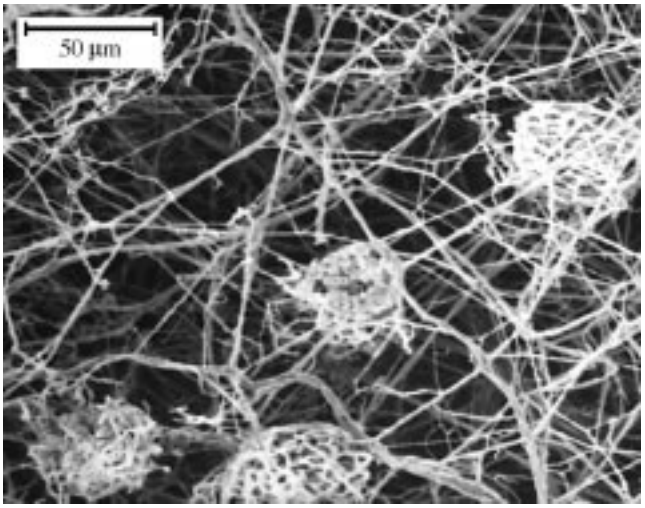

Figure 2. *Gymnascella marismortui*. Barely differentiated ascomata (scanning electron micrograph).

3. RESULTS AND DISCUSSION

Fifteen samples out of several hundred tested gave rise to growth of fungal mycelia. Three species of filamentous fungi were identified from Dead Sea surface waters, one belonging to the Ascomycota, and two species belonging to the Deuteromycota. The ascomycete (seven isolates) was identified as a new species belonging to the genus *Gymnascella* (*Gymnoascaceae*), here described as *G. marismortui* sp. nov. The Deuteromycota were identified as *Ulocladium chlamydosporum* Mouch. (Hyphomycetes) (three isolates) and *Penicillium westlingii* Zaleski (Anamorphic Trichocomaceae) (five isolates).

Gymnascella marismortui Buchalo, Nevo, S. Wasser, Oren, et Molitoris, sp. nov. (figures 1–6).

Colonies 45–50 mm diam. post 18 dies 26 °C, optime 25–28 °C rescentes. Hyphis vegetativus levibus, septatus, tenuiter tunicatus, 1.1–1.6 μm diam. Et hyphis 3.0–5.0 μm diam. Incrassatus ad 7–10 μm diam. Arthroconidiis levibus, tenuiter tunicatus, 3.0–5.0 μm × 2.0–3.8 μm. *Chlamydosporiis 5.0–10* μm diam. Ascocarpis discretis presentibus, sphericis, 40–70 μm diam., albis. Compositis peridii hyalinis septatis. Hyphis sterilibus 2.0–2.5 μm et hyphis raynet presentibus ad 7–10 μm in diam. Ascis sphericis 9.3–13.3 μm diam, hyalinis, octosporis, evanescentibus. Ascosporis sphericis, ellipsoideis, 5.3–6.6 μm × 3.3–4.7 μm, chryseis, verruceis.

Typus: Isolata e aqua maris mortui prope Ein-Zukim in Israel. Cultura in Instituto Evolutionensis, Univ. Haifa (UHA) sub # 1632 et in Instituto Botanici Kiewiensis (KW) sub # 2005 conservatur.

Etymology: marismortui—of the Dead Sea.

Colonies on malt agar with 50% Dead Sea water, cottony, initially white, then light brown; sporulating areas farinaceous, granular, first white, later yellowish. Colonies at 26–28 °C attained 45–50 mm in diam. in 15–18 days. At 37 °C, growth is slow and restricted. Reverse yellowish or light reddish-brown. Vegetative hyphae smooth, septate, thin-walled, 1.1–1.6 μm wide. Also present, hyphae with nodulose roughened swellings up to 7–10 μm diam. at crosswalls, mycelial cords 50–60 μm

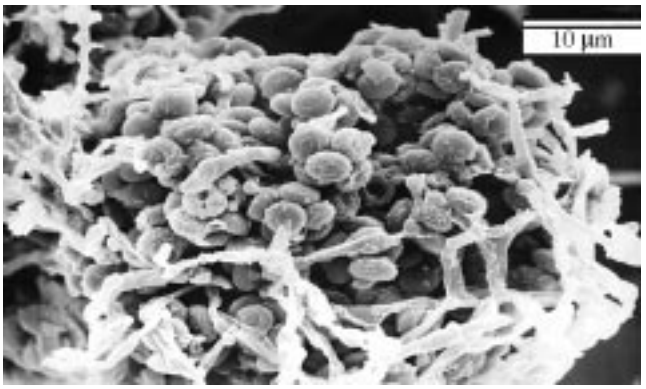

Figure 3. *Gymnascella marismortui*. Asci surrounded by peridial hyphae (scanning electron micrograph).

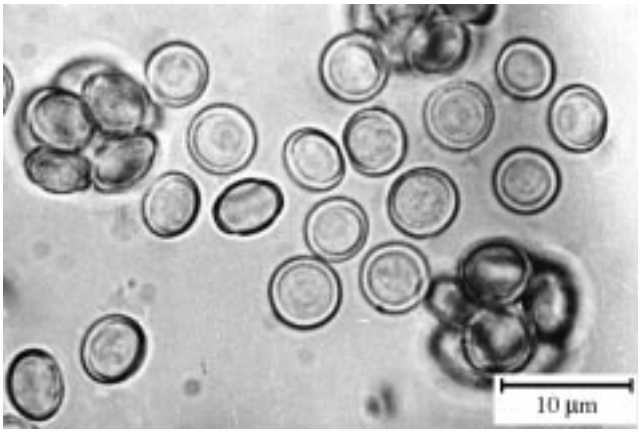

Figure 4. *Gymnascella marismortui*. Asci and ascospores (differential interference contrast microscopy, ×1000).

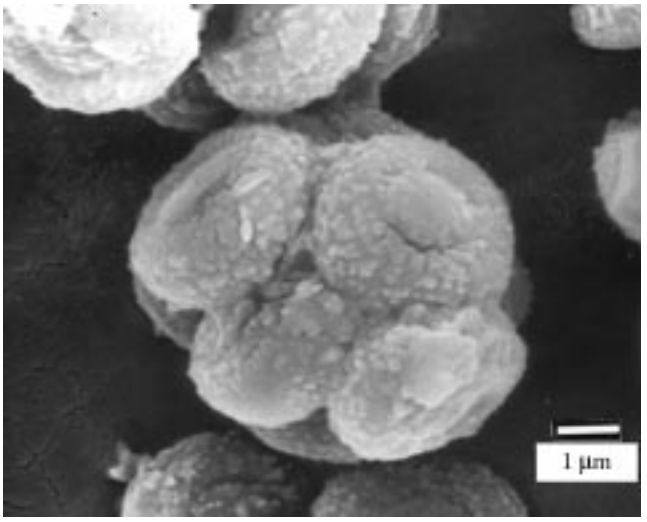

Figure 5. *Gymnascella marismortui*. Asci and ascospores (differential interference contrast microscopy, ×1000).

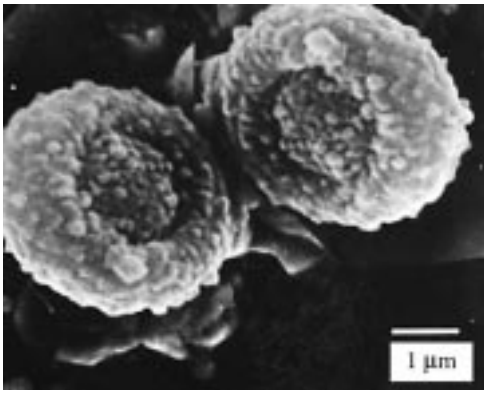

Figure 6. *Gymnascella marismortui*. Ascospores (scanning electron micrograph).

wide (figure 1*a*,*c*). Anamorph: smooth thick-walled arthroconidia 2.0–2.8 µm × 3–5 µm, and solitary or catenalate intercallar chlamydospores 5–10 µm in diam. (figure 1*b*,*d*). Ascomata barely differentiated, white, spherical, 40–70 µm in diam., consisting of clusters of ascospores surrounded by vegetative mycelium (figures 2 and 3). The size of the ascomata was determined when mature ascospores were present after two, four and six months of storage of cultures on agar media. Ascal groups are held together loosely by hyphal strands up to 5 µm wide, peridial hyphae resembling vegetative hyphae, 2.0–2.5 µm wide (figure 3). Asci (figures 4 and 5) are spherical or ellipsoidal, eight-spored, 9.3–13.3 µm in diam., average 10.8 µm (n=100), hyaline, walls evanescent. Ascospores (figures 4–6) pale yellow, spherical in front view, 5.3–6.6 µm in diam. (n=100) and ovate in side view, 3.3–4.7 µm wide, with a broad equatorial rim 1.3–2.2 µm (n=100). The central part of the ascospore is separated from the periphery by a deep furrow. Differential interference contrast microscopy and scanning electron microscopy shows verrucose appearance of the ascospore surface (figure 6).

Material examined: Israel, Dead Sea, near Ein-Zukim, isolated from Dead Sea water, UHA 1632 and KW 2005.

Affinitas: G. marismortui differs from all known species of the genus *Gymnascella*, including *G. punctata* (Dutta et Ghosh) Currah, by the shape of its ascospores, which have a very broad rim (Currah 1985). The shape of ascospores is an important taxonomic characteristic in the genus *Gymnascella*. *G. marismortui* also differs from *G. punctata* by the presence of arthroconidia and chlamydospores, the presence of nodulose hyphae, the colour and much smaller size of the ascomata (*ca.* 250 µm in *G. punctata* compared with 40–70 µm in *G. marismortui*), and obligate halophily.

Characteristics of the Dead Sea isolates of *U. chlamydosporum* and *P. westlingii* correspond to those described in literature (Mouchacca 1971; Ellis 1976; Pitt 1979; Ramirez 1982; Domsch *et al*. 1993; Gams 1993).

All three fungi grew and sporulated on agar with 50% Dead Sea water. *Gymnascella marismortui* was shown to be an obligate halophile, growing optimally in the presence of 0.5–2 M NaCl or 10–30% (by volume) Dead Sea water. High sucrose concentrations supported only slow growth. The occurrence of viable spores of filamentous fungi in the Dead Sea and their ability to grow and sporulate on nutritional media with high concentrations of Dead Sea water suggest that these fungi may be able to live in the Dead Sea as vegetative mycelium. They may occur on some substrates, such as plant residues and wood, at least during the rainy period or in diluted

areas near freshwater springs. Fungal hyphae were identified on wood constructions in areas of diluted Dead Sea water. Final confirmation of vegetative growth of these species in the Dead Sea awaits future experiments *in vivo* and in the laboratory, using optimized liquid media with undiluted Dead Sea water. Agar media were unsuitable for the purpose, because of crystal formation when the Dead Sea water concentration exceeded 50%.

Investigations of the *Penicillium westlingii* and *Ulocladium chlamydosporum* isolates showed that both are salt tolerant in the salinity range from 3 to 15% at 26 °C. The cultures investigated grew well and sporulated at these conditions. After a short adaptation (one transfer) on media with 50% Dead Sea water, growth was obtained on all media. However, on malt agar media with 50% Dead Sea water, mycelial growth and sporulation were poor compared with 15% salinity artificial seawater media.

Out of 33 water samples of 2 ml from different depths (between 10 m and 310 m, which is close to the bottom), 16 gave rise to fungal growth, and at least some of the isolates may represent new species. These will be reported elsewhere.

The data reported in this paper indicate that filamentous fungi may play a hitherto overlooked role in the food web of the Dead Sea. The relatively low pH of the Dead Sea water (around 6) makes the Dead Sea a potentially suitable habitat for fungal life, but the salinity of the water may inhibit growth. The same is true for other forms of life inhabiting the Dead Sea, namely the green alga *Dunaliella* (Oren & Shilo 1985), and the most magnesium-tolerant species among the halophilic Archaea (Mullakhanbhai & Larsen 1975). Only a significant dilution of the upper water layers of the Dead Sea enables the biota to overcome inhibition by the exceedingly high salt concentrations (and specifically the high concentrations of the divalent cations, magnesium and calcium). It is unknown to what extent the blooms of algae and Archaea that occurred in the lake in 1980 and in 1992 (Oren 1988, 1993) were accompanied by an increase in fungal biomass and activity. However, the finding that some of our fungal isolates grew in the presence of 50% Dead Sea water, as do the other microorganisms isolated from the lake, demonstrates that they may be adapted to life in the extremely stressful, hypersaline Dead Sea water.

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REFERENCES

Adler, L. 1996 Fungi and salt stress. In *Fungi and environmental change* (ed. J. C. Frankland, N. Magan & G. M. Gadd), pp. 217–234. Cambridge University Press.

Amon, J. P. 1978 Thraustochytrids and Labyrinthulids of terrestrial, aquatic and hypersaline environments of the Great Salt Lake, USA. *Mycologia* **70**, 1299–1301.

Andrews, S. & Pitt, J. I. 1987 Further studies on the water relation of xerophilic fungi, including some halophiles. *J. Gen. Microbiol.* **133**, 233–238.

Blomberg, A. & Adler, L. 1993 Tolerance of fungi to NaCl. In *Stress tolerance of fungi* (ed. D. H. Jennings), pp. 209–232. New York: Marcel Dekker, Inc.

Brown, A. D. 1990 *Microbial water stress physiology. Principles and perspectives*. Chichester, UK: Wiley.

Cronin, A. E. & Post, F. J. 1977 Report of a dematiaceous Hyphomycetes from the Great Salt Lake, Utah. *Mycologia* **69**, 846–847.

Currah, R. S. 1985 Taxonomy of the Onygenales: Arthrodermataceae, Gymnoascaceae, Myxochytridiaceae and Onygenaceae. *Mycotaxon* **24**, 1–216.

Domsch, K. H., Gams, W. & Anderson, T. H. 1993 *Compendium of soil fungi*. Eching: IHW-Verlag.

Elazari-Volcani, B. 1940 Studies on the microflora of the Dead Sea. PhD thesis, The Hebrew University of Jerusalem. [In Hebrew.]

Ellis, M. B. 1976 *More dematiaceous Hyphomycetes*. Kew: Commonwealth Mycological Institute.

Gams, W. 1993 *Supplement and corrigendum to the compendium of soil fungi*. Eching: IHW-Verlag.

Hammond, P. M. 1995 Practical approaches to the estimation of the extent of biodiversity in speciose groups. In *Biodiversity measurement and estimation* (ed. D. L. Hawksworth), pp. 119–136. London: Chapman & Hall.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. & Pegler, D. N. 1995 *Ainsworth & Bisby's dictionary of fungi*, 8th edn, pp. 1–616. Wallingford, UK: CAB International.

Javor, B. 1989 *Hypersaline environments—microbiology and biogeochemistry*. Berlin: Springer.

Jones, E. B. G. 1988 Do fungi occur in the sea? *The Mycologist* **2**, 150–157.

Kohlmeyer, J. & Kohlmeyer, E. 1979 *Marine mycology. The higher fungi*. New York: Academic Press.

Kritzman, G. 1973 Observations on the microorganisms in the Dead Sea. MSc thesis, The Hebrew University of Jerusalem. [In Hebrew.]

Krumgalz, B. S. & Millero, F. J. 1982 Physico-chemical study of the Dead Sea waters. I. Activity coefficients of major ions in Dead Sea water. *Mar. Chem.* **11**, 209–222.

Kushner, D. J. 1978 Life in high salt and solute concentrations: halophilic bacteria. In *Microbial life in extreme environments* (ed. D. J. Kushner), pp. 171–215. New York: Academic Press.

May, R. M. 1988 How many species are there on earth? *Science* **241**, 1441–1449.

May, R. M. 1995 Conceptual aspects of the quantification of the extent of biological diversity. In *Biodiversity measurement and estimation* (ed. D. L. Hawksworth), pp. 13–20. London: Chapman & Hall.

Mouchacca, D. J. 1971 Une nouvelle espece du genre *Ulocladium* Preuss. *Rev. Mycol.* **36**, 114–122.

Mullakhanbhai, M. F. & Larsen, H. 1975 *Halobacterium volcanii* spec. nov., a Dead Sea halobacterium with a moderate salt requirement. *Arch. Microbiol.* **104**, 207–214.

Oren, A. 1988 The microbial ecology of the Dead Sea. In *Advances in microbial ecology* (ed. K. C. Marshall), pp. 193–229. New York: Plenum Press.

Oren, A. 1992 Ecology of extremely halophilic microorganisms. In *The biology of halophilic bacteria* (ed. R. H. Vreeland & L. I. Hochstein), pp. 25–53. Boca Raton: CRC Press.

Oren, A. 1993 The Dead Sea—alive again. *Experientia* **49**, 518–522.

Oren, A. & Shilo, M. 1985 Factors determining the development of algal and bacterial blooms in the Dead Sea: a study of simulation experiments in outdoor ponds. *FEMS Microbiol. Ecol.* **31**, 229–237.

Pitt, J. I. 1979 *The genus* Penicillium *and its telemorphic states* Eupenicillium *and* Talaromyces. London: Academic Press.

Ramirez, C. 1982 *Manual and atlas of the Penicillia*. Amsterdam: Elsevier Biomedical.

Rohrmann, S., Lorenz, R. & Molitoris, H. P. 1992 Use of natural and artificial seawater for investigation of growth, fruit body production, and enzyme activities in marine fungi. *Can. J. Bot.* **70**, 2106–2110.

Volcani, B. 1944 The microorganisms of the Dead Sea. In *Papers collected to commemorate the 70th anniversary of Dr Chaim Weizmann*, pp. 71–85. Rehovoth: Daniel Sieff Research Institute.

Wilkansky, B. 1936 Life in the Dead Sea. *Nature* **138**, 467.

Wilson, E. O. 1992 *The diversity of life*. Cambridge, MA: Harvard University Press.